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<p>13. ABSTRACT (Maximum 200 Words) During the third year we have focused on making significant progress in both the MRI and the MRS portions of this project. We have been hampered by difficulty in recruiting suitable subjects and by difficulty in getting female subjects to return for a second MRS study. Nevertheless we have confirmed that 1) Women recruit their upper bodies more than men, and 2) Quadriceps glycogen is nominally depleted by our protocol in both genders. We are now able to consider several additional preliminary conclusions. 1) As prolonged exercise continues muscle recruitment patterns do not change. 2) The left biceps muscle is more heavily recruited in women (MRI) and this results in faster glycogen depletion rates (MRS) in women than in men. 3) Net liver glycogen depletion rates during exercise are slower in women in the luteal phase of their menstrual cycle than in men or in women in the follicular phase. This suggests that hormonal fluxes exert an effect under our experimental conditions. We have begun a second MRS study on the effect of four consecutive days of our protocol and early data suggest that in both genders exercised muscles progressively supercompensate glycogen. The major failure of this current project is the paucity of suitable subjects that has resulted in our low numbers of completed studies to date. We are considering increasing subject compensation, and we intend to continue the project beyond term in order to confirm or refute our conclusions thus far.</p>			
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FOREWORD

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TABLE OF CONTENTS

I.	Front Cover	p.1
II.	SF 298	p.2
III.	Foreword	p.3
IV.	Table of Contents	p.4
V.	Introduction	pp.5-7
VI.	Body of Report	pp.8-22
VII.	Key Research Accomplishments	pp.22-23
VIII.	Reportable Outcomes	p.24
IX.	Conclusions	p.24
X.	References	p.24
XI.	Figure Legend	pp.25-26
XII.	Figures	pp.27-39

INTRODUCTION

The third year of this project has been focused in three major areas: A. Completing the MRI portion of the project, B. Continuing progress in the MRS portion of the project and beginning the second study of this part of the research, and C. Improving the effectiveness of our subject recruitment process. In the first major area we had five goals: 1) Complete the data acquisition phase of the MRI study [see Year 1 & Year 2 reports (1,2)]. 2) Complete a comparative analysis of muscle recruitment patterns brought about by the initial exercise bout in the male and female subject groups (1,2). 3) Complete an analysis of any changes in muscle recruitment patterns that occur as the prolonged exercise protocol proceeds (1,2). 4) Identify the muscles that are most significantly recruited by the exercise protocol and compare the degree of recruitment of these muscles in the male and female subject populations. 5) Prepare a manuscript for publication. In the second major area we had six goals: 6) Complete the first MRS study in a significant number of male and female subjects. 7) Complete the first MRS study during both menstrual phases in a significant number of female subjects. 8) Identify male/female and/or female menstrual differences, if any, in muscle and/or liver glycogen depletion patterns. 9) Begin the second MRS study and determine whether four consecutive days of the prolonged exercise protocol is feasible. 10) If the protocol is feasible, complete at least two studies and determine whether there is a discernable glycogen depletion/recovery pattern. 11) Decide whether the final MRS study, carbohydrate supplementation, is worthwhile. In the third area we had two goals: 12) Increase the number of minority subjects studied. 13) Increase the total number of subjects recruited and improve the recruitment efficiency.

In our first principal area (goal 1) we have completed the data acquisition portion of the MRI study (6M & 6F). In this study 100% of male subjects and 33% of female subjects were able to complete the entire (12 blocks of 15min exercise) protocol (1,2). The data analysis is split so as to allow us to distinguish any differences in muscle recruitment (male versus female) brought about by performing the lift-and-carry exercise (goal 2), and to determine whether the continuation of prolonged exercise

over 12 15min blocks causes any change in muscle recruitment (goal 3). In other words, as the exercise protocol progresses do some muscles fatigue and other muscles take over to insure that the task is accomplished? Data analysis (goal 2 & goal 3) has been tedious, requiring 50-80 man-hours per subject, and is not yet complete. Goal 2 (analysis of initial bout of exercise) is 75% (9-of-12 studies) completed, whereas goal 3 (analysis of prolonged exercise) is only 33% (4-of-12 studies) completed. From the MRI data we have been able to identify the muscles that are consistently recruited in both sexes by our protocol (Goal 4), and we have identified muscles that are recruited to a significantly greater extent in the female population (Goal 4). We are not yet ready to prepare the manuscript (Goal 5); however, preliminary preparation has begun, and we expect to submit an abstract to a national meeting within the next month. We have been working on some more sophisticated ways of rendering the MRI data, and we have made some progress in the area that will be discussed later in this report. We feel that as more of the data are analyzed the findings will show conclusive differences between the men and women who performed this task. Furthermore, while it is still not complete, we feel that a significant amount of progress has been made in this area during the past year.

In our second major area of focus, the first goal (goal 6) was to complete a significant number of studies on the first MRS study. We have made progress on this goal; however, I would characterize the results as less than successful so far. We have now completed 18 total studies (9 M, 5 F mid-luteal, and 4 F mid-follicular). As we discussed in last years report (2), it has been difficult to recruit and complete subjects and we continue to examine the problem. Another problem that has arisen is a difficulty in getting female subjects, examined in one phase of their menstrual cycle, to return for the other phase. The intention of the original proposal was to study these women in paired fashion; however, if we cannot resolve this latest difficulty we may have to consider performing this portion of the project as an unpaired study. Although luteal/follicular female subjects have been difficult (goal 7), we have been able to complete enough studies for a marginal comparison of the two phases. We feel that goal 6 and goal 7 have been only marginally successful. Because of the small number complete studies in the two menstrual phases, goal 8 has

also been only marginally successful. However, there are enough data to suggest that we will be able to draw some definite conclusions about female menstrual cycle effects when a few more studies are completed.

We have successfully begun the second MRS study and have determined that it is feasible for our subjects to perform the prolonged exercise protocol during four consecutive days (goal 9). We have completed the 4da protocol in three subjects (2 M, 1 F), and have seen a distinct glycogen depletion/recovery pattern (goal 10). We feel that goal 9 and goal 10 have been accomplished. Based on MRS data from the first and second MRS studies we have successfully accomplished goal 11, determining that, based on the glycogen recovery patterns following our prolonged exercise protocol, the final carbohydrate supplementation protocol would not be a worthwhile pursuit. We have verbally communicated this to the COR for this project, and are forwarding a written confirmation. In the third principal area of focus this year we have been unsuccessful in both goals. We have failed to complete any studies involving minority subjects (goal 12); however, we currently have a tentative agreement with three black subjects (1 M, 2 F) to participate. Goal 13 has been completely unsuccessful; our efficiency in subject recruitment and retention has declined during the past year.

During year three this project has made significant progress; however, we have had only marginal success in accomplishing the goals for the year. The result is that the project is currently not on track with the revised SOW that was agreed upon at the end of year 1. The primary reason for this is difficulty in recruiting and retaining suitable test subjects. On the positive side, because we are not completing the predicted number of studies, we are currently under-spending the contract, which opens the door for a non-funded extension after the term of the contract. Nonetheless, when all things are considered, the data that have been generated are clear and we are being led to definite conclusions. Therefore, I would have to say that even though the number of completed studies is not where we would like it to be the project has been successful to this point.

BODY OF REPORT

A. Experimental methods, assumptions, and procedures:

The experimental methods, assumptions, and procedures have been largely described in the first two annual reports (1,2). This section focuses primarily on the continuation of experiments that have been described in these earlier reports (1,2).

Complete the MRI portion of the project – Although this area is not complete, we have made significant progress in this area of the project. Each of the individual goals of this area is presented below.

1. Complete data acquisition of MRI study – The data acquisition phase of the MRI study has been completed; six male and six female subjects have been studied. The experimental protocol (exercise and MR imaging protocol) has been described in earlier annual reports (1,2). The bruising on the legs and thighs of our female subjects has been minimized by the addition of padding on the corners of the lift-and-carry box.
2. Comparative analysis of muscle recruitment patterns brought about by the initial bout of exercise (male versus female groups) – We have completed this analysis in 5 male and 4 female subjects (75% of total). Based upon the data analyzed thus far, we have determined that gender differences do exist in muscle recruitment patterns brought about by 15min (45 lifts) lift/carry exercise. The MRI analytical techniques employed in our data analysis are described in the year 2 annual report (2).
3. Comparative analysis of muscle recruitment pattern changes during the course of prolonged exercise. We have completed analysis in 1 male and 3 female subjects (33% of total), employing the same analytical techniques as in goal 2 (2). WE have focused on the female population, reasoning that their smaller body size would provide the greatest likelihood of detecting alterations in muscle recruitment patterns over the course of the prolonged protocol. We have been unable to detect any change in muscle recruitment pattern over the

course of the prolonged protocol, as compared with the initial 15min of exercise.

4. Identify the muscles that are most significantly recruited – Our data analysis thus far has demonstrated that muscles in the upper legs are consistently recruited in both sexes. Furthermore, muscles in the upper body, particularly the biceps, trapezius, and deltoid muscles are recruited to a significantly greater extent in the female population. In addition to identifying gender specific muscle recruitment patterns, this goal was intended to identify the muscles or groups of muscles best suited for MR spectroscopy. This goal has been successfully accomplished during the past year, and it is anticipated that completion of the analysis in the remainder of subjects will only serve to tighten the data.
5. Prepare a manuscript for publication – This goal has not been accomplished. Although we have begun to prepare the manuscript, the final version will not be written until the remainder of data analysis is complete.

The second major area of focus during the past year was to make a significant amount of progress in the first MRS study and to begin the second MRS study of this portion of the project. While subject recruitment continues to be difficult, we have had a great deal of success in this area during the past year. The individual goals are described below.

6. Complete the first MRS study in a significant number of male and female subjects – We have completed 18 total MRS studies (9 male and 9 female), and have gathered enough data to allow us to begin to see muscle and liver glycogen depletion patterns. The MRS acquisition and analysis techniques employed in this portion of the project have been described in the year 2-report (2). The MRS study has been expanded to include the quadriceps muscle group, the left biceps muscle and the liver. The quadriceps are studied because this muscle group is consistently recruited in both genders, while the left biceps muscle is studied because our MRI has revealed that it is significantly more recruited in the female population. The number of liver glycogen measurements has been reduced. It is intended that the liver

glycogen measurements be used to verify that large net changes in liver glycogen are not brought about by the prolonged protocol. Liver glycogen turnover would be an interesting measurement that would provide much more information about systemic carbohydrate balance during our extended exercise protocol. However, turnover measurements are much more difficult, and invasive to the extent that a clamp protocol (infusion) is involved. While more studies will be required to complete this portion of the project, a significant amount of progress has been made over the past year.

7. Complete a significant number if studies during both menstrual phases in the female population – Of the 9 female studies that we have completed 5 have been mid-luteal and 4 have been mid-follicular. This goal has had only marginal success, owing largely to difficulty in getting the female subjects to return for a second study. To date we have been committed to conducting the menstrual cycle effects portion of the project as a paired study. We are currently re-examining the protocol to determine whether an unpaired would work well enough to provide a viable alternative. Of the female subjects studied, three who have agreed to return for a second study have not yet returned. We are currently attempting to schedule these studies; however, these subjects have moved away from the New Haven area (all as students) and we will have to try and get them when they are home from school. The analysis of venous blood samples has not been completed as of this report.
8. Identify male/female and/or female menstrual differences, if any, in muscle and/or liver glycogen depletion patterns – We have identified several trends in muscle and liver glycogen depletion during the prolonged lift & carry protocol. The quadriceps glycogen depletion rate is not as fast in the female/mid-luteal group as in the male subjects, while the female/mid-follicular group lies between the other two subject populations. The data are not yet significant; however, the trend suggests that with additional studies we will observe both gender and menstrual cycle differences in quadriceps glycogen depletion rates (Figure 7). The biceps glycogen depletion rates are faster in both female populations as compared with the male population.

Again, the trend suggests that with additional studies we will see a significantly greater biceps glycogen depletion rate in the female population, and no menstrual cycle effect (Figure 7). This trend, which is different from the trend in the quadriceps, suggests that the female population must work their biceps muscles harder than the males, whereas the quadriceps muscles are not working as hard. We found that the net liver glycogen depletion rate during the exercise protocol is significantly slower in the female/mid-luteal group than in the male or the female/mid-follicular populations (Figure 8). While we will need to increase the number of studies to establish significance in the muscle measurements, the data thus far suggest that both menstrual cycle and gender differences do exist. Our 4.7T 30cm spectrometer is not appropriate for the biceps or the quadriceps muscles because the bore size permits only the lower limb into the magnetic center (calves, forearms). Therefore we have performed all studies on our 2.1T 100cm spectrometer, which does not have the interleaved C-13/P-31 capability (the ability to acquire two different spectra during a single time period). The necessity to perform individual acquisitions at the C-13 and the P-31 frequencies has led us to forego P-31 measurements in favor of C-13. Therefore, we have not obtained data on P-31 metabolites so far. To perform these additional acquisitions would alter the prolonged exercise protocol in such a way as to make the spectral acquisition break (between blocks of exercise) too long. We have investigated both the anterior forearm and the gastrocnemius and found the quadriceps and biceps to be of far more interest. Therefore, we have no additional P-31 data to report at this time.

9. Begin the second MRS protocol and determine the feasibility of four consecutive days of the prolonged lift & carry protocol – This is the only new study to be initiated during year 3 of this project. The experimental methods are almost identical to the first MRS study, with the exception that subjects were allowed to exercise in thirty minute blocks and there were no MR measurements acquired during the breaks. MR spectra were acquired only before and immediately after the complete protocol. This study was initiated

with the assumption that four consecutive days of the lift & carry protocol would produce measurable effects on muscle glycogen depletion and recovery patterns. We have completed the four-day protocol in 2 male subjects and 1 female subject. The male subjects were able to complete the protocol in each of the four days, whereas the female subject did not perform the complete protocol on any of the four days. However, she was able to perform the protocol longer during each consecutive session of the four-day protocol. We have determined that the four-day protocol is feasible and that the protocol yields interesting data.

10. If the four-day protocol is feasible, complete at least 2 studies and identify glycogen depletion/recovery patterns – This goal has been successfully accomplished. Over the four-day protocol subjects' dietary intake has been held to an iso-caloric mixed-meal plan. We have identified an interesting glycogen recovery pattern in the quadriceps and the biceps muscles whereby the muscles progressively supercompensate glycogen over the four days of the study.
11. We have observed nominal amounts of glycogen depletion in the liver, and in the individual muscles that we have studied (quadriceps and biceps). These observations, combined with the progressive glycogen supercompensation seen in the four-day protocol with an iso-caloric mixed-meal diet, indicate that an MRS study of carbohydrate supplementation is probably not a worthwhile investment of our resources. We feel that the resources are better invested in the studies that are currently underway, and should be applied toward increasing the number of ongoing studies. This has been discussed with our COR, and we have drafted a letter describing the proposed changes to the SOW.

The final major area of focus during the past year has been to improve our performance in the area of subject recruitment. We have had little success in this area over the past year, and we continue to struggle with subject recruitment.

12. Increase the number of minority subjects studied – We have studied no minority subjects to date; however, we do have recruitment conversations

open with several minority subjects (2 black male, 1 black female, and 1 Hispanic male).

13. Increase the total number of subjects recruited and improve the recruitment efficiency – We have failed to improve on either of these goals.

B. Results:

1. Completion of the MRI portion of the project – These results, although not thoroughly analyzed, have revealed significant gender differences in muscle recruitment patterns. We have examined fifty different muscles (25 right and 25 left) in our male and female subject populations (analysis 75% complete), studying the effect of the initial 15min bout of lift and carry exercise. We have seen that, in the upper body the chest (pectoralis major and pectoralis minor) and the triceps muscles were not significantly recruited in either gender. In the trunk the rectus abdominus muscles were not significantly recruited in either gender. The lower back muscles are recruited in both genders; however, those muscles are on the edge of the field-of-view of the MR imager and therefore have not been readable in all studies. We hope that when the final 25% of subjects are analyzed we will have sufficient numbers to include the lower back muscles in our data set. All other muscles have been included in the data set presented in this report.

When the all other muscles examined in this study were grouped and compared according to gender, there was a significant difference ($p<0.000001$) in total body muscle recruitment between men and women. The mean T_2 increase following 15min of exercise, presented in Figure 1, was 3.9 ± 0.2 msec in the male population and 2.5 ± 0.2 msec in the female population. Muscles were then grouped according to the upper and lower regions of the body. In the lower body (pelvis, thighs, lower legs) the mean T_2 increase following 15min of exercise was not significantly different between genders (3.3 ± 0.4 msec in males and 3.9 ± 0.3 msec in females) (Figure 2). However, the upper body showed significant differences between genders (1.8 ± 0.5 msec in males and 4.0 ± 0.6 msec in females, $p<0.005$) (Figure 2). Muscles were then assessed according to muscle group, and the data are presented in Figure 3.

There were significant differences between genders in all muscle groups of the upper body. In the upper arms (biceps, deltoids) the mean T_2 increase following 15min of exercise was 1.6 ± 0.5 msec in men and 3.8 ± 0.6 msec in women ($p < 0.01$). The triceps are not included in this comparison because they were not recruited in either gender. In the forearms (anterior and posterior forearm) the mean T_2 increase following 15min of exercise was 3.1 ± 0.7 msec in men and 5.2 ± 0.7 msec in women ($p < 0.005$). In the upper back (trapezius, latissimus dorsi) the mean T_2 increase following 15min of exercise was 0.7 ± 0.4 msec in men and 3.1 ± 0.5 msec in women ($p < 0.001$) (Figure 3). In the lower body there were significant differences between genders in the thighs but not in the lower legs (Figure 3). In the quadriceps muscle group the mean T_2 increase following 15min of exercise was 2.9 ± 0.4 msec in men and 3.8 ± 0.3 msec in women ($p < 0.005$). In the hamstrings group the increases were 3.8 ± 0.6 msec in men and 4.7 ± 0.7 msec in women ($p < 0.05$). In the triceps surae (medial & lateral gastrocnemius, soleus), and in the gluteus group (gluteus maximus, medius, minimus) there were no significant differences between genders.

Individual muscle pairs (right and left) were grouped and compared according to gender. There were no significant differences between gender for all muscle pairs in the lower body (Figure 4,5). In the upper body there were gender differences in the biceps ($p = 0.0013$), the deltoids ($p = 0.0363$), the anterior forearm ($p = 0.0065$), the posterior forearm ($p = 0.0006$), the trapezius ($p = 0.0005$), and the latissimus dorsi ($p = 0.0171$) (Figure 6). Finally, individual muscles were compared for gender differences. There were significant gender differences in two individual muscles of the upper body the left biceps ($p = 0.0363$) and the left trapezius ($p = 0.0025$). Because we observed a trend toward greater recruitment of the left side of the body (due to the design of the exercise apparatus that requires a higher lift by the left side), we compared the left and right side muscles. There were no significant differences between the left and right sides of the body in either gender, nor in both genders grouped together. We have completed the analysis of the entire exercise protocol in four subjects. Thus far we have observed no changes in muscle recruitment patterns for the fifty individual muscles tested. The preliminary assumption that as the subjects began to fatigue they would recruit different muscles may prove to be untrue.

2. Continuing progress on the MRS portion of the project – These results are still inconclusive owing to the small number of studies completed to date. However, there are several interesting trends that point to potentially significant final conclusions. From the 18 studies completed thus far, we have compared 9 male subjects with 5 female subjects in the luteal phase of their menstrual cycle and 4 female subjects in the follicular phase. We have measured glycogen depletion patterns in the left quadriceps muscles (vastus lateralis and vastus intermedius) and in the left biceps brachii (biceps and brachialis) muscles. We have also measured net glycogen changes in the liver during the prolonged lift and carry protocol.

We have observed a trend toward higher glycogen depletion rates in the left quadriceps muscles of the male population (-5.8 ± 1.4 mmol/l-hr) than in the female population (-4.1 ± 1.9 mmol/l-hr, follicular), particularly in the luteal phase of the menstrual cycle (-1.6 ± 3.3 mmol/l-hr, luteal) (Figure 7). The variability of this measurement suggests that we would require 35 studies in each group to observe significant differences between the male and female/luteal populations, and 50 studies (each group) would be required to detect significant differences between the male and female/follicular groups. To detect menstrual cycle differences in an unpaired female population >100 studies would be required. Therefore, it does not seem reasonable to predict that this project will reveal significantly higher quadriceps glycogen depletion rates in men than in women, nor will it reveal menstrual cycle differences in an unpaired female population.

In the left biceps muscle the trend goes the other way with higher glycogen depletion rates in the female population (both menstrual phases) (-9.9 ± 3.0 mmol/l-hr, luteal and -9.5 ± 3.9 mmol/l-hr, follicular) than in the male population ($+2.4 \pm 3.5$ mmol/l-hr) (Figure 7). Unfortunately, we have studied only 2 male, 2 female/luteal, and 4 female/follicular subjects. This portion of the project was initiated later based on data from the MRI study that found the greatest gender differences in muscle activity in the left biceps. The variability of this measurement so far suggests that we will be able to detect significant differences between men and women in the luteal phase with $n=8$ in both groups. It will require 14 studies (each group) to detect differences between men and women in the follicular phase of their menstrual cycle.

Based on this analysis it seems reasonable to predict that we will be able to detect significant gender differences in glycogen depletion rates in the left biceps during the course of this project. However, it does not seem reasonable to predict that we will detect menstrual cycle differences in an unpaired female population.

When net liver glycogen depletion rates were assessed during the lift and carry protocol a significant difference was observed between the male population (-0.146 \pm 0.018mmol/l-min) and the female/luteal population (-0.092 \pm 0.016mmol/l-min) ($p=0.0439$). This data are presented in Figure 8. There were no differences between men and women in the follicular phase of their menstrual cycle (-0.149 \pm 0.024mmol/l-min), nor were there menstrual cycle differences. Based on the variability of these measurements 16 studies will be required to detect significant menstrual cycle differences in an unpaired population of female subjects. Therefore, it seems reasonable to predict that we will be able to establish both gender differences and menstrual cycle differences in net liver glycogen depletion rates during exercise over the course of this study.

We have begun a second MRS study to examine the effect of four consecutive days of our prolonged lift and carry exercise protocol upon glycogen depletion/recovery patterns in the left quadriceps and left biceps muscles. The preliminary results of this study, presented in Figure 9, suggest that in both muscles there is a progressive supercompensation of glycogen, with an iso-caloric mixed-meal diet that was administered to each subject. This suggests that a high carbohydrate diet is not required to produce supercompensation with consecutive days of this type of exercise. Mean glycogen in the quadriceps was 115.4 \pm 20.1mmol/l before exercise on day 1 of the protocol and rose to 135.2 \pm 21.3mmol/l (118%) before exercise on day 4 of the protocol (Figure 9). Mean glycogen in the biceps was 65.6 \pm 8.2mmol/l (day1, before) and rose to 90.7 \pm 11.5mmol/l (138%) before exercise on day 4. These results are preliminary; however, they suggest that 14 studies will be required to detect significant differences in resting biceps glycogen by day 4, and 16 studies to detect differences by day 3. However, based on the variability seen thus far 90 studies would be required to detect differences in resting quadriceps glycogen by day 4. We have not obtained enough data to predict whether we will see any gender differences;

however, the four-day glycogen depletion/recovery patterns were similar in all subjects studied so far.

C. Discussion:

During the third year of this project we have focused on collecting enough data to allow us to draw some conclusions about gender differences during our prolonged exercise protocol. In the MRI portion of the project we have observed significantly greater whole body muscle recruitment in the female population (Figure 1). When the muscle analysis was grouped according to upper and lower body it was revealed that the greater muscle recruitment in the female population was primarily in the upper body. The degree of muscle recruitment, measured as the T_2 increase from resting to exercised state, is an indication of how hard the muscle is working. Therefore, a similar T_2 increase in the lower body and pelvis would indicate that both male and female populations are working their lower bodies to a similar degree. The quadriceps group has been consistently activated in all subjects, leading to the decision to study this muscle group with MR spectroscopy. The greater T_2 increases seen in the upper bodies of the female population indicates that women must work their upper bodies harder to perform the same task. As we indicated in last year's report, this is likely a function of muscle mass and height.

We examined muscle groups in the upper body, the pelvis, and in the lower body and observed significantly greater muscle recruitment in the upper arms (without the triceps), forearms, and upper backs of the female population. We observed no gender differences in muscle recruitment of the pelvic area. However, the quadriceps and the hamstrings of the female population were recruited to a significantly greater extent in the female population. No gender differences were observed in the lower legs. These observations indicate that, while the lower body as a whole does not exhibit gender differences, the female population does work their thighs harder than the male population. Muscle group differences in T_2 increases remained greater in the upper body. When individual pairs of muscles (right and left) were assessed no gender differences were observed in the pelvis, thighs, or lower legs. However, the female

population recruited 6 muscle pairs of the 11 pairs tested in the upper body. Again, this indicates that the female population needs to work their arms (biceps, deltoids, anterior and posterior forearms) harder to accomplish the same task as the men, and they work their trapezius and latissimus dorsi muscles harder. Because this protocol requires that the shorter female subjects lift the 65lb box above their shoulders, subject height may play a significant role in these findings. We have attempted to recruit short male subjects and tall female subjects with only marginal luck. However, if we can generate a pool of short men and tall women we will be able to address the question of gender effects versus height effects.

Although this report does not present T₂ data in an image format, we have made a significant amount of progress toward ultimately rendering T₂ data overlaid on reconstructed 3-dimensional images. These 3-D images are reconstructs of a series of approximately 180 transverse 2-D images, and can be presented in any orientation desired. When rendering of the total data set is interrupted at some point the image appears as if the body was sliced open at that point and all underlying tissues, including muscles, become visible. Figure 10, Figure 11, and Figure 12 represent a 3-D reconstruction without T₂ data overlays. These reconstructs are rendered with three possible data set interruptions, demonstrating three possible views of the underlying tissue. When this image presentation method is fully developed we will be able to focus on any area of the total musculature to demonstrate exercise induced T₂ data, which occurs as increased signal. This presentation technique will allow us to present this very complicated data set in a simple manner that readily demonstrates variations in total body muscle recruitment according to gender, workload, type of exercise, or any number of variables that we may choose to manipulate. We would also note that the lower leg muscle recruitment study that was referenced in the first two yearly reports (1,2) has been submitted for publication in the Journal of Applied Physiology. Although that study is not a part of the work funded in this project, it has a bearing on the ultimate publication of our muscle recruitment MRI study in that it establishes the validity of MRI as a quantitative measurement by comparison with EMG.

We have undertaken the first MRS study in order to examine gender and menstrual cycle differences in two specific muscles/ muscle groups. The vastus

lateralis and vastus intermedius muscles are recruited to a similar degree in both genders. If the MRI data were a reliable indication of the underlying metabolism, we would expect to see a similar degree of glycogen depletion in these two muscles. The development of MRI as a predictor of MRS assessed metabolism has not been demonstrated, and therefore is of considerable importance. The trend that we have observed in the quadriceps MR spectra suggests that the women are able to carry out the task while conserving glycogen. However, the variability observed thus far indicates that we will not be able to establish significance. These results suggest that our MRI data has successfully predicted the metabolic outcome of the prolonged lift and carry protocol. If the variability improves with more studies, we may see a difference in men versus women in the luteal phase of their menstrual cycle. This would suggest that at such a low workload the women burn fat more efficiently, a reasonable conclusion given the increased availability of intramuscular fat normally seen in women. The quadriceps glycogen depletion rates observed in this study were compared with rates seen in earlier work in this lab (3). Our previous studies involved a plantar flexion protocol that exercised a single muscle (gastrocnemius) so that the workload could be quantified (3). The current protocol is different in that it requires systemic exercise and the contribution to each muscle to the total work performed is unknown. Therefore, we do not know the workload of the individual muscles studied in this project. However, if we assume that the glycogen depletion rate is dependent upon the workload, we can get an idea of the workloads being performed in this project. Based upon the glycogen depletion rates seen in this year's data, we estimate the male and female left vastus lateralis and vastus intermedius to be working at somewhere between 10% and 15% of their maximum voluntary contraction (MVC). Glycogen depletion rates from our earlier work are presented in Figure 13.

Data on the biceps suggest that, while the men are not working their left biceps muscles, the women are working their left biceps muscles at a higher rate than their quadriceps muscles. A comparison with our previous data (Figure 13) suggests approximately 20% of MVC. The biceps data are less reliable because of the small number of studies completed so far. However, the variability thus far indicates that

we will be able to establish a significantly greater rate of left biceps glycogen depletion in the female population as compared with the males. Again, this is in agreement with the prediction of our MRI data that demonstrates greater recruitment of the left biceps in the female population. Muscle glycogen measurements in both muscle studied indicate that glycogen is not severely depleted in either gender with our prolonged lift and carry protocol.

The net liver glycogen measurements obtained thus far indicate a difference between the male population and the female/luteal population. The liver measurements are complicated by the liver's ability to turnover its glycogen stores so that blood glucose concentrations can be maintained. Muscles do not have the enzyme necessary to cleave the terminal phosphate group from glucose-6-phosphate (glucose-6-phosphatase) and allow the release of glucose from muscle cells. The liver also has the ability to rapidly synthesize glycogen from three-carbon compounds generated by muscle metabolism and taken up by the liver (gluconeogenesis). Therefore, our measurements cannot track turnover and only indicate the net liver glycogen concentration at the time of measurement. However, by obtaining these net liver glycogen measurements we can get some sense of the systemic carbohydrate balance that is moderated by the liver. Our data indicate that the prolonged lift and carry exercise protocol does not significantly challenge the liver's ability to maintain readily available carbohydrate stores. Our initial results also suggest that normal female hormonal variations may have some effect on liver glycogen metabolism during prolonged exercise.

We have initiated a second MRS study to examine the effect of performing our prolonged lift and carry exercise protocol on four consecutive days. Although not much data is in thus far, the preliminary results suggest that the glycogen depletion/recovery pattern generated by this protocol will be interesting. Preliminary data suggests a trend toward progressive supercompensation as the protocol continues over four days. This supercompensation occurs in the absence of a high carbohydrate diet, indicating that the body can completely recover from the protocol overnight and prepare for the next day's demands. We do not yet have enough data to compare

genders; however, the data that we do have suggest similar patterns in the different genders.

D. Relationship to the Statement of Work (SOW) outlined in the proposal:

The administration of this grant continues to suffer from a difficulty in recruiting appropriate test subjects in the private sector. When compared with both the original SOW and the revised SOW, we are currently lagging behind in all areas of the project. Fortunately, all experimental procedures that have been undertaken have produced interesting results. Because we have not completed the number of studies that were originally intended at this point, we are under-spending our grant to a great extent. When the number of studies proposed versus the remaining term of the contract, it is clear that more time will be required to complete the project as originally intended. However, because we have under-spent we intend to continue accumulating studies beyond the term of the grant until we have reached definite conclusions in all phases of the project. Based on the results thus far, it is also possible that fewer studies will be required in order to complete the aims of the project. Following telephone conversations with our COR, we have drafted a letter indicating our intent to exclude the nutritional supplement study. As of this date, we have begun all other phases of the project and it is our intention to continue accumulating data in these areas as we can recruit suitable test subjects. The necessity to convert the female menstrual cycle portion of the project may necessitate additional studies, which will be funded by resources from the nutritional supplement study. It is also possible that we may need to increase the subject reimbursement in order to attract a greater number of potential subjects. This decision will be made through conversations with our COR and the Human Investigations Committee here at Yale.

E. Negative results:

We have discovered that our 4.7T spectrometer will be unavailable for the study of secondary muscles. This is because of our decision to study muscle of the upper leg and the upper arm, muscles that cannot be studied in the small bore system. This has necessitated that all studies be performed in our whole body 2.iT spectrometer which does not have the capability for interleaved C-13/P-31 spectroscopy. We have attempted to perform additional acquisitions on the 2.1T system; however, the time required to collect the data interferes with the exercise protocol to the extent that the studies become impossible to perform. For this reason, we have abandoned the P-13 measurements in all studies performed thus far. It is likely that these measurements will not be picked up in future studies, thereby eliminating the P-31 portion of the project.

F. Problems in accomplishing tasks:

The problems that were reported in the year 2 annual report (2) have persisted during the past year, and continue to slow the project. The problem of subject recruitment is particularly bothersome. We will continue to recruit as many subjects as we can with the intention of continuing our studies beyond the term of the contract. Blood sampling and processing problems with our GCRC and dietary regulation problems also continue to persist.

KEY RESEARCH ACCOMPLISHMENTS

Year 1:

- Developed an echo-planar MRI protocol
- Designed and constructed a lift & carry exercise apparatus

Year 2:

- Demonstrated that MRI is capable of detecting gender differences in the lift & carry task
- Demonstrated that MRI can point to the muscles best studied with MRS
- Determined that liver glycogen is not significantly depleted by the lift & carry protocol in either gender
- Determined that quadriceps muscle glycogen is nominally depleted by the lift & carry protocol in both genders

Year 3:

- Demonstrated with MRI that women performing the lift & carry protocol recruit their upper body muscles, particularly arms, to a greater extent than do men
- Demonstrated with MRI that as the prolonged lift & carry protocol continues the whole body muscle recruitment pattern remains the same as following the initial exercise period
- Demonstrated with MRI that the quadriceps muscles are consistently recruited by the lift & carry protocol in both genders
- Demonstrated with MRI that the biceps muscles are recruited to a significantly greater extent by the lift & carry protocol in women
- Determined that the biceps would provide the greatest opportunity to demonstrate metabolic gender differences during the lift & carry protocol using MRS
- Demonstrated with MRS that, during lift & carry exercise, women in the luteal phase of their menstrual cycle deplete liver glycogen at a significantly slower rate than do men.
- Determined that with the current variability gender differences in biceps glycogen depletion rates will be demonstrated in this project

REPORTABLE OUTCOMES

1999: (July) Women's Health Research Workshop Series, National Center of Excellence in Women's Health at Yale. Title: "Equality in the Military: Will All Jobs be Open to Women?" [PRESENTATION]

CONCLUSIONS

From this third year of our project we conclude that women recruit their upper bodies, particularly their arms, to a greater extent than men. Furthermore, the muscle recruitment pattern does not change as the exercise session progresses. Hence, new muscles are not recruited to compensate for fatiguing muscles. Net female liver glycogen depletion rates during the exercise protocol are significantly reduced in the luteal phase of their menstrual cycle, suggesting a hormonal effect upon exercising liver carbohydrate metabolism. Biceps glycogen depletion rates during the lift and carry protocol may be faster in women than in men; however, more studies are needed to establish significance. If this holds true it will represent the first time that MRS has been used to confirm MRI as a means of predicting muscle glycogen metabolism. Finally, when more studies are completed we predict that we will be able to conclude that muscles exercised on several consecutive days glycogen load in order to prepare for anticipated metabolic demands. We believe that this will hold true in both genders.

REFERENCES

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2. Price, T.B. Year 2 annual report for Contract Number: DAMD17-96-C-6097.
3. Price, T.B., et al. *J.Appl.Physiol.* 70(4): 1836-1844, 1991.

FIGURE LEGEND

1. Average T₂ change [msec] calculated from all muscles that were significantly recruited. Female subjects recruited to a significantly greater extent ($p<0.000001$). male n=5, female n=4, total number of muscles assayed = 38
2. Average T₂ change [msec] calculated from the upper body and the lower body of male (n=5) and female (n=4) subject populations. Upper body muscles assayed = 12, lower body muscles assayed = 26
3. A. Average T₂ change [msec] calculated from the three major muscle groups in the upper bodies of men (n=5) and women (n=4). Upper arms = 4, forearms = 4+, and upper back = 4. B. Average T₂ change [msec] calculated from the three major muscle groups in the lower bodies of men (n=5) and women (n=4). Quadriceps = 8, hamstrings = 6, and triceps surae = 4. * $p<0.05$, ** $p<0.005$, *** $p<0.001$.
4. A. Average T₂ changes [msec] calculated from muscle pairs in the thighs. B. Average T₂ changes [msec] calculated from muscle pairs in the lower leg. 11 pairs studied, represents 22 individual muscles (right & left sides).
5. Average T₂ change [msec] calculated from the three major muscle pairs of the pelvis (6 individual muscles studied).
6. A. Average T₂ changes [msec] calculated from five muscle pairs in the arms and shoulders. The triceps muscles were not significantly recruited by the exercise protocol. * $p<0.05$. B. Average T₂ changes [msec] calculated from six muscle pairs in the trunk. The pectoralis major & minor and the rectus abdominus muscles were not significantly recruited by the exercise protocol. The lower back is significantly recruited in the female population; however, with n=1 in the male population we probably will not be able to compare genders for these muscles. * $p<0.05$.
7. A. Quadriceps glycogen depletion rates during the lift & carry protocol in 9 males, 5 luteal females, and 4 follicular females. B. Biceps glycogen depletion rates during the lift & carry protocol in 9 males, 5 luteal females, and 4 follicular females.

8. Liver glycogen depletion rates during the lift & carry protocol in 9 males, 5 luteal females, and 4 follicular females. $P=0.0439$ for males versus mid-luteal females.
9. A. Quadriceps glycogen concentrations before and after exercise on four consecutive days for three subjects (2 male, 1 female). B. Quadriceps glycogen concentrations before and after exercise on four consecutive days for three subjects (2 male, 1 female).
10. 3-D image reconstruction of a series of transverse images. Series is interrupted in the transverse plane at mid thighs.
11. 3-D image reconstruction of a series of transverse images. Series is interrupted in the coronal plane at body center.
12. 3-D image reconstruction of a series of transverse images. Series is interrupted in the sagital plane at mid-leg left of body center.
13. Gastrocnemius glycogen depletion rates for single-muscle plantar flexion exercise at three different workloads (ref 3). 10% MVC n=5, 15 % MVC n=9, 20% MVC n=5.

Figure 1:

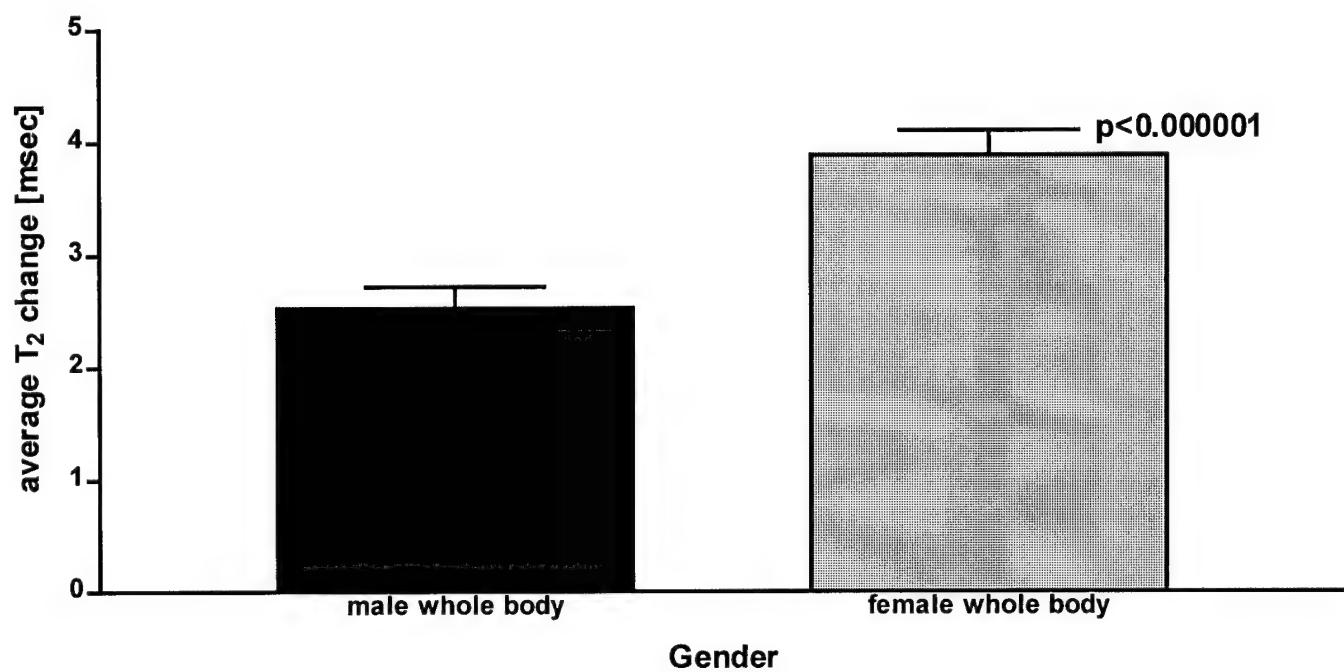


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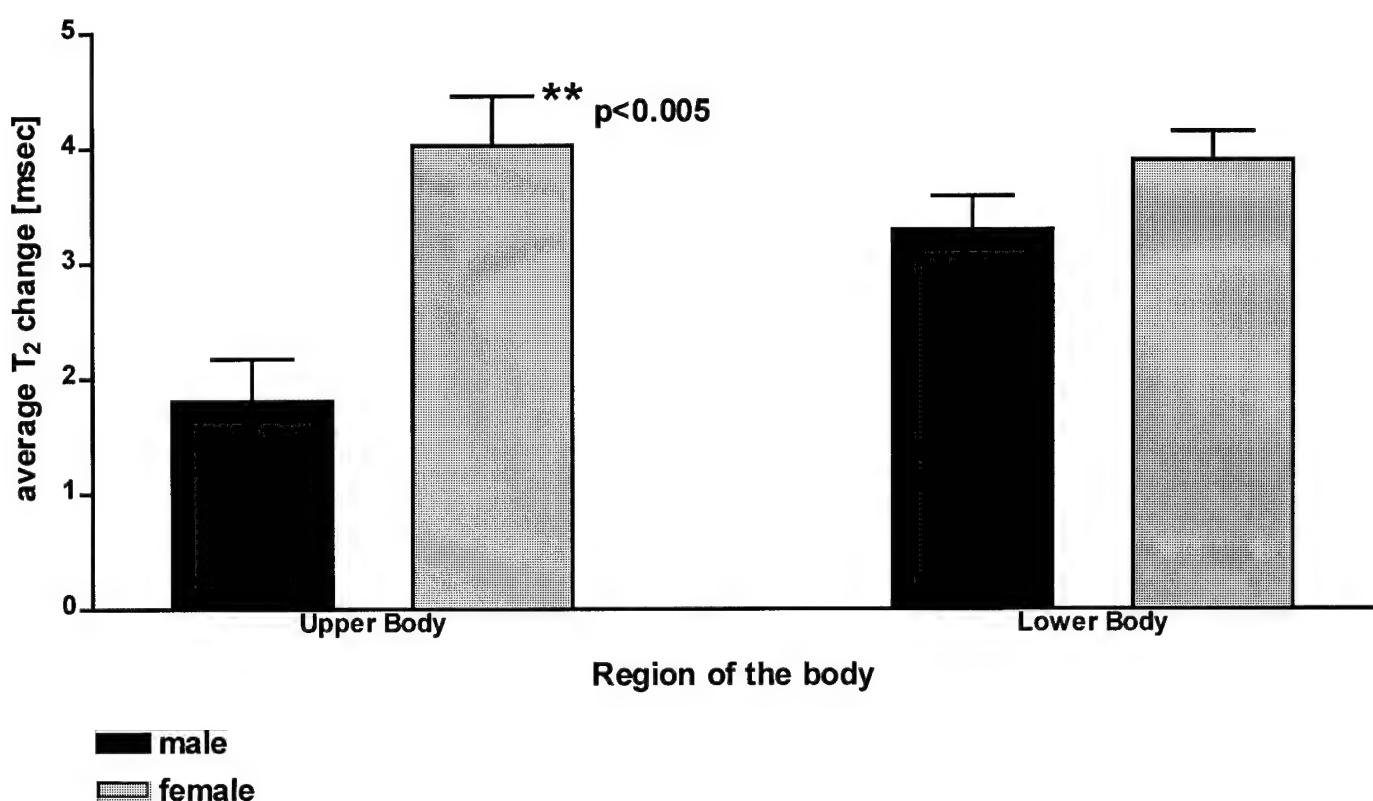
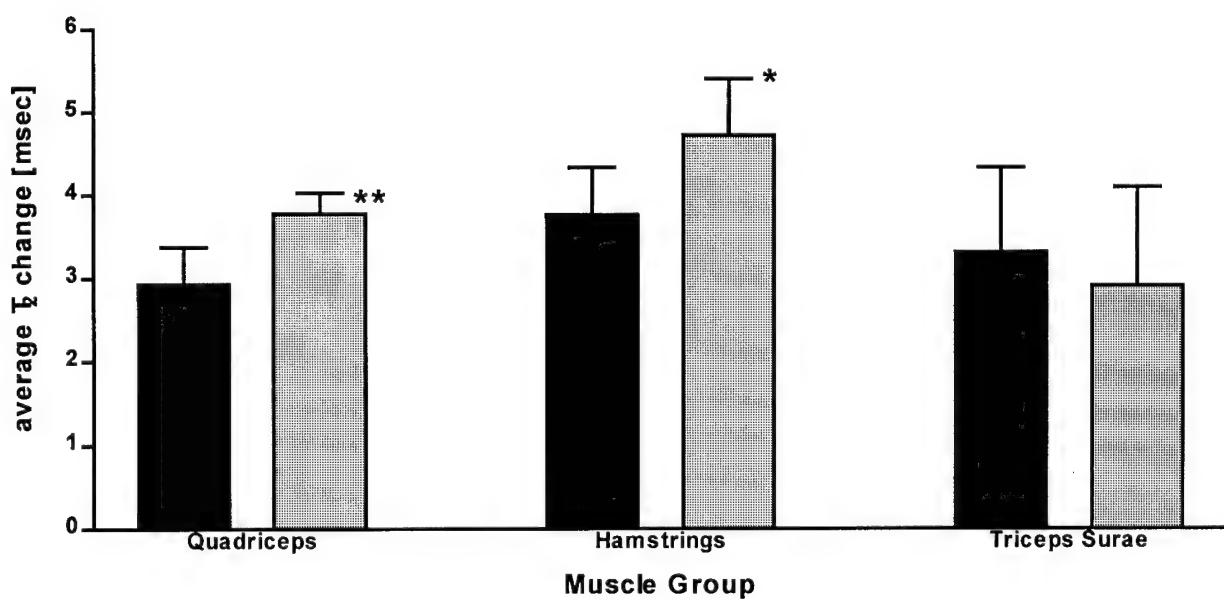
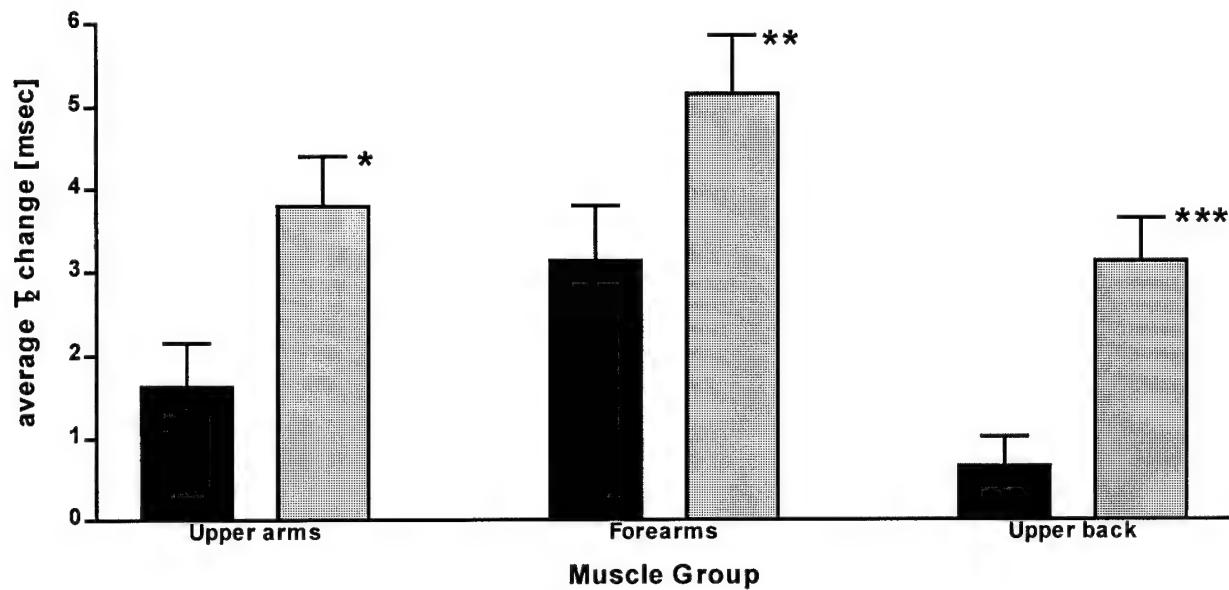


Figure 3:



** $p < 0.005$ * $p < 0.05$

█ male

█ female

Figure 4:

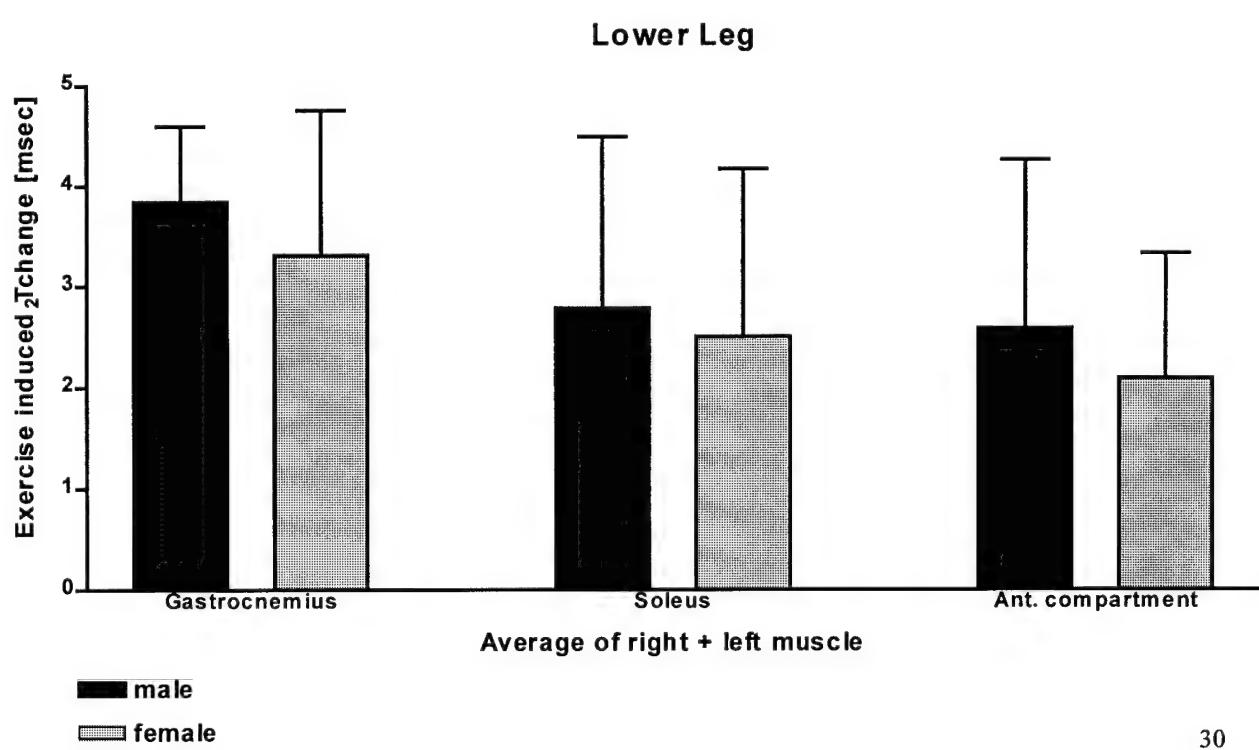
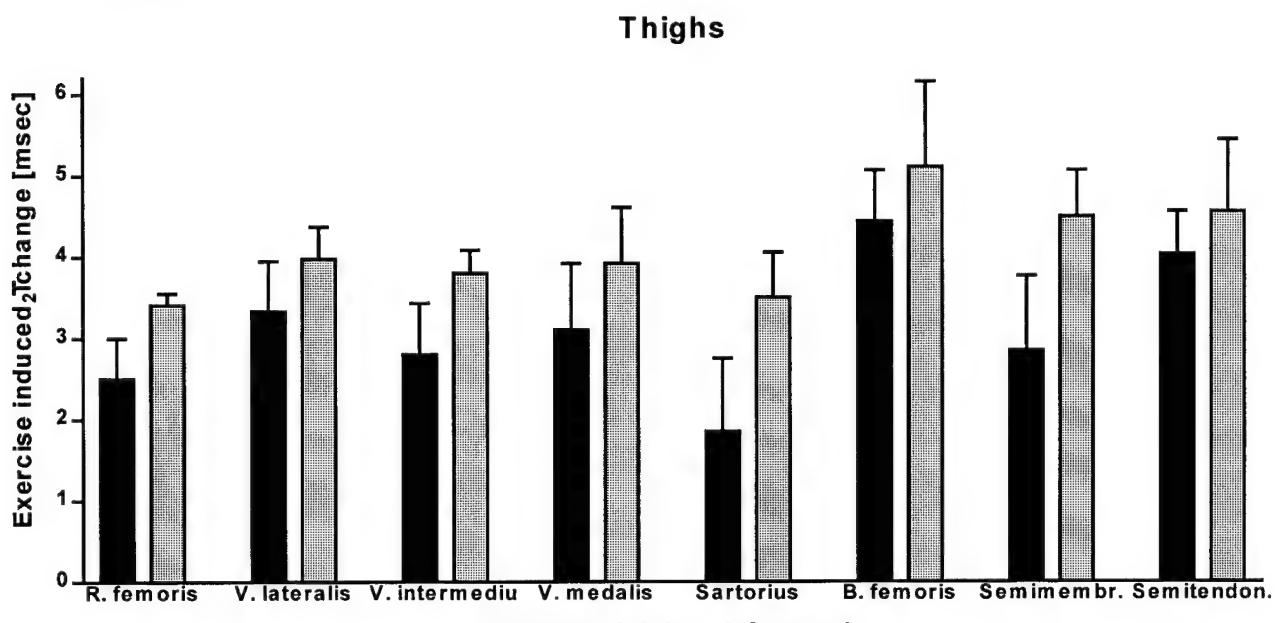


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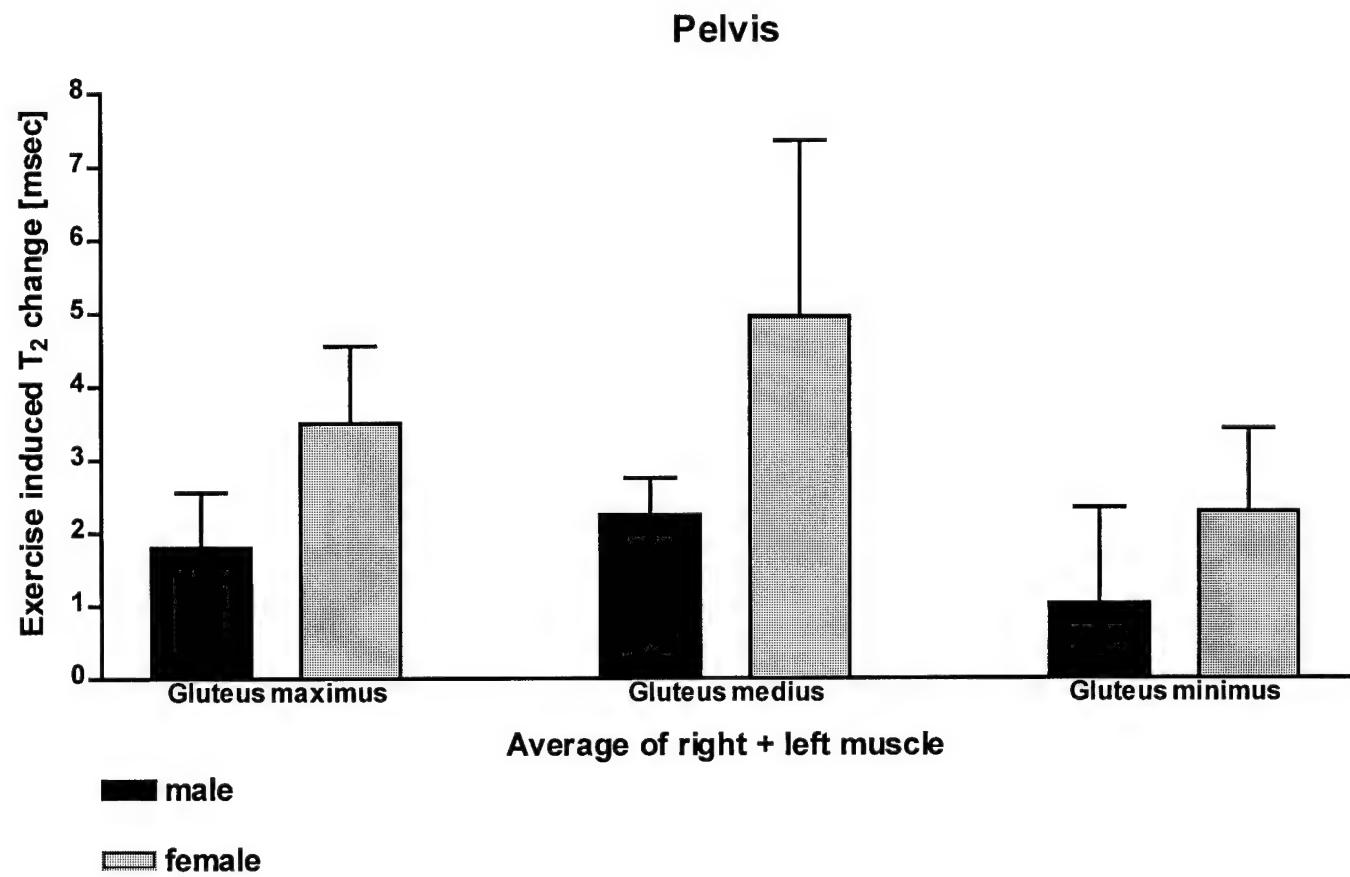
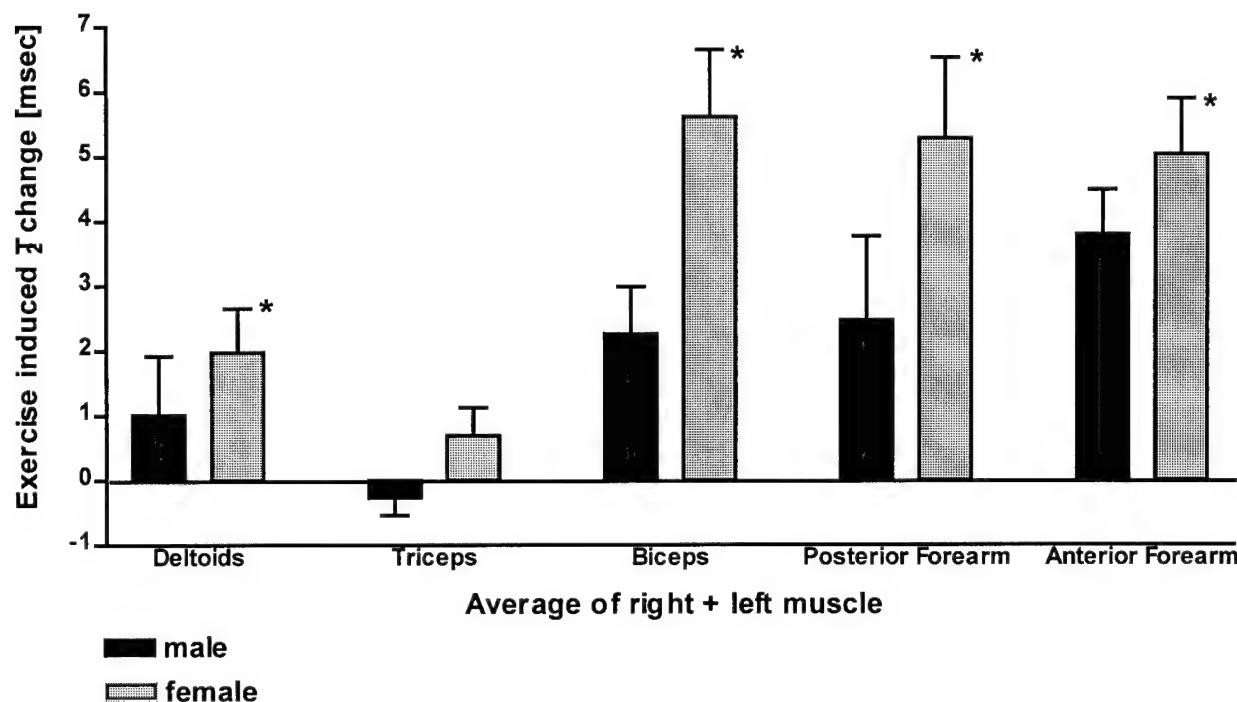


Figure 6:

ARMS & SHOULDERS



Trunk

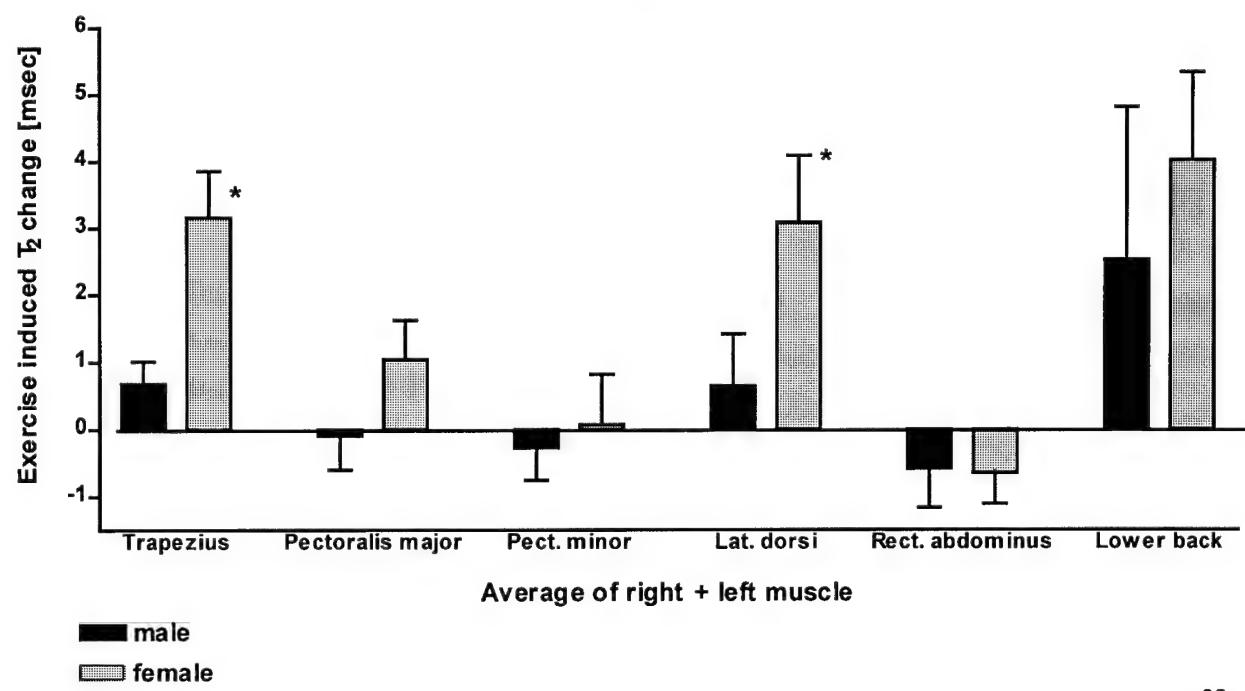


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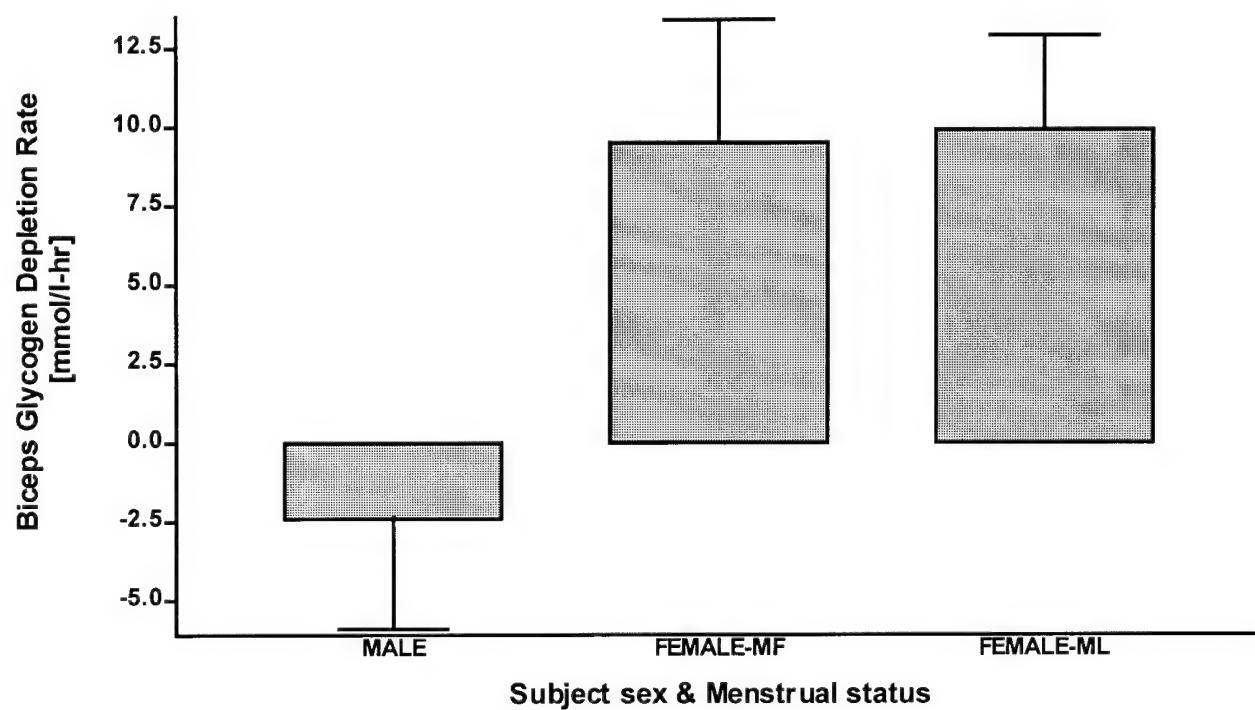
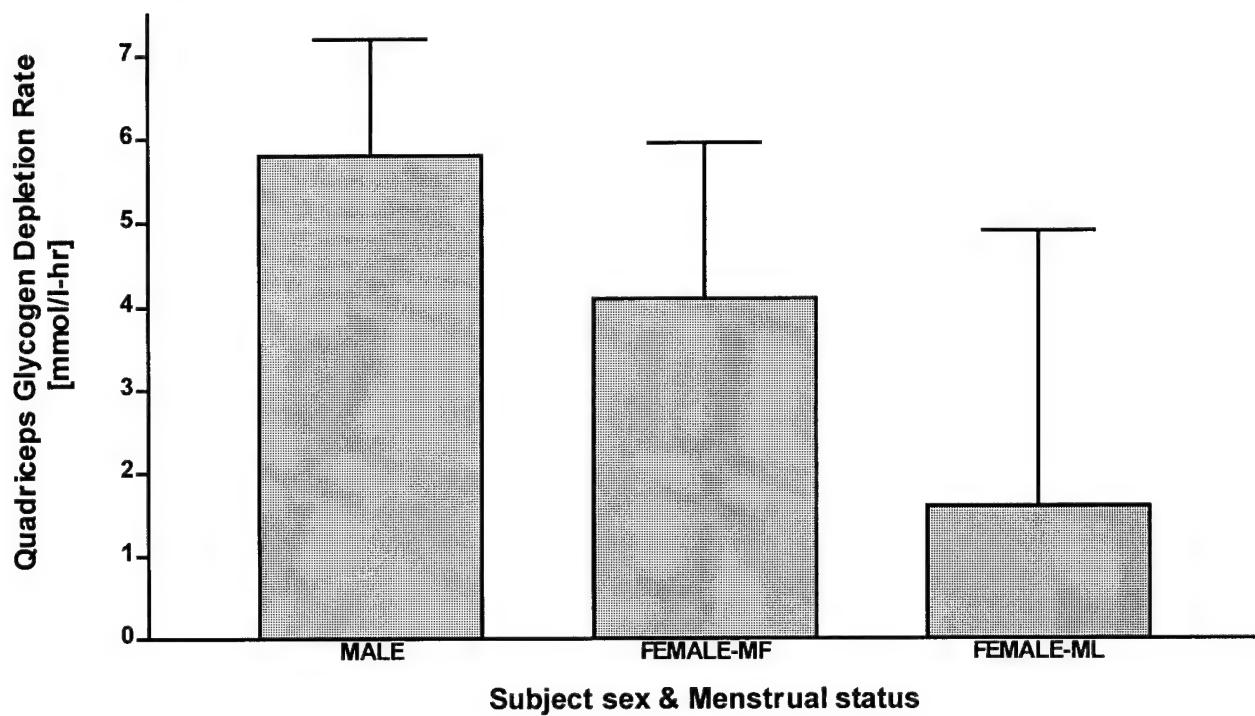


Figure 8:

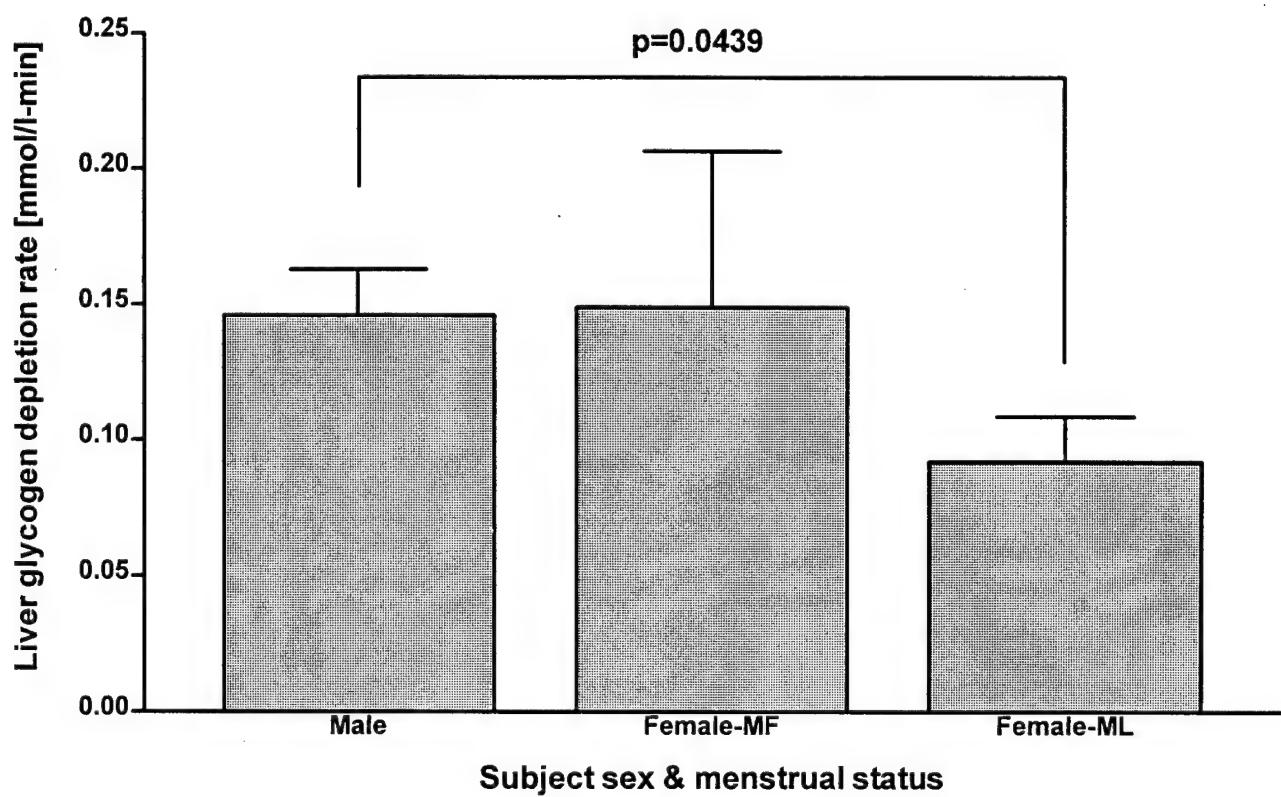


Figure 9 :

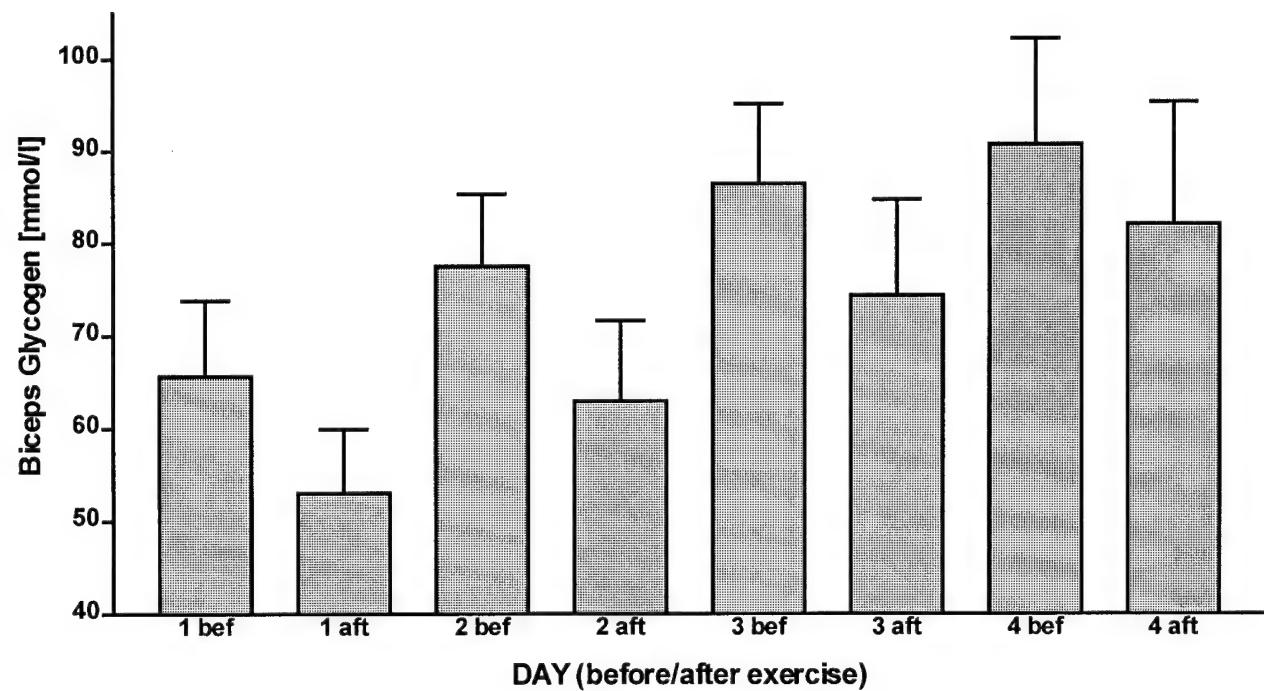
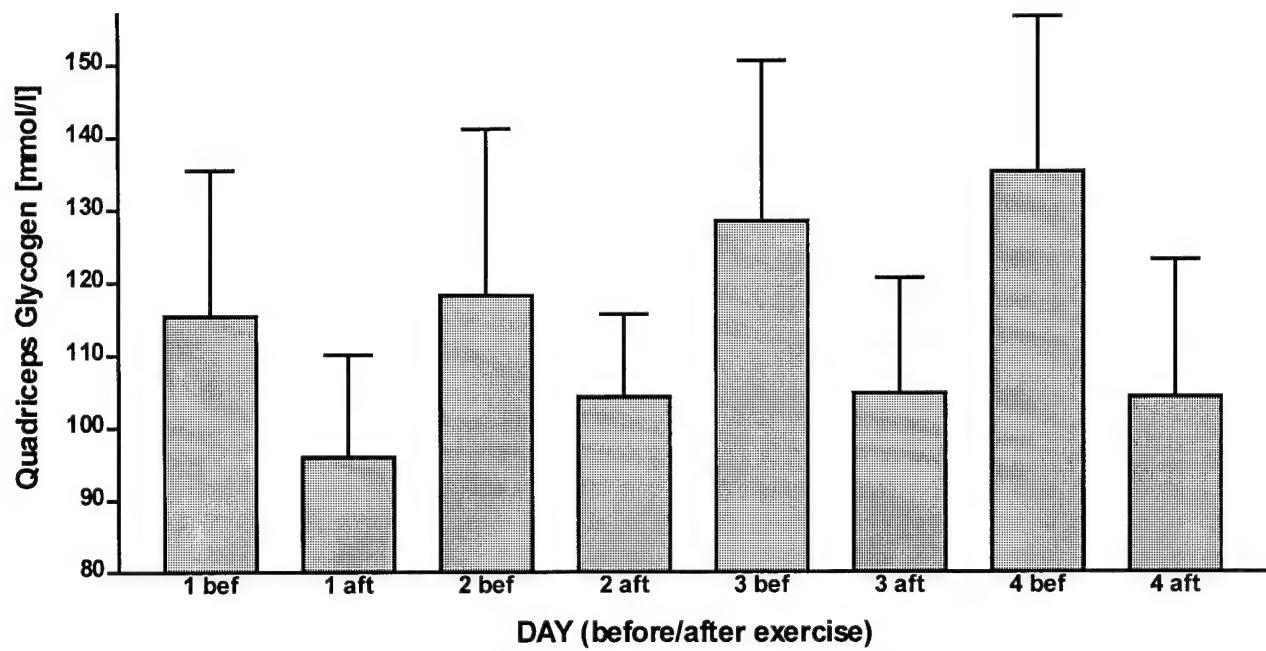


Figure 10:

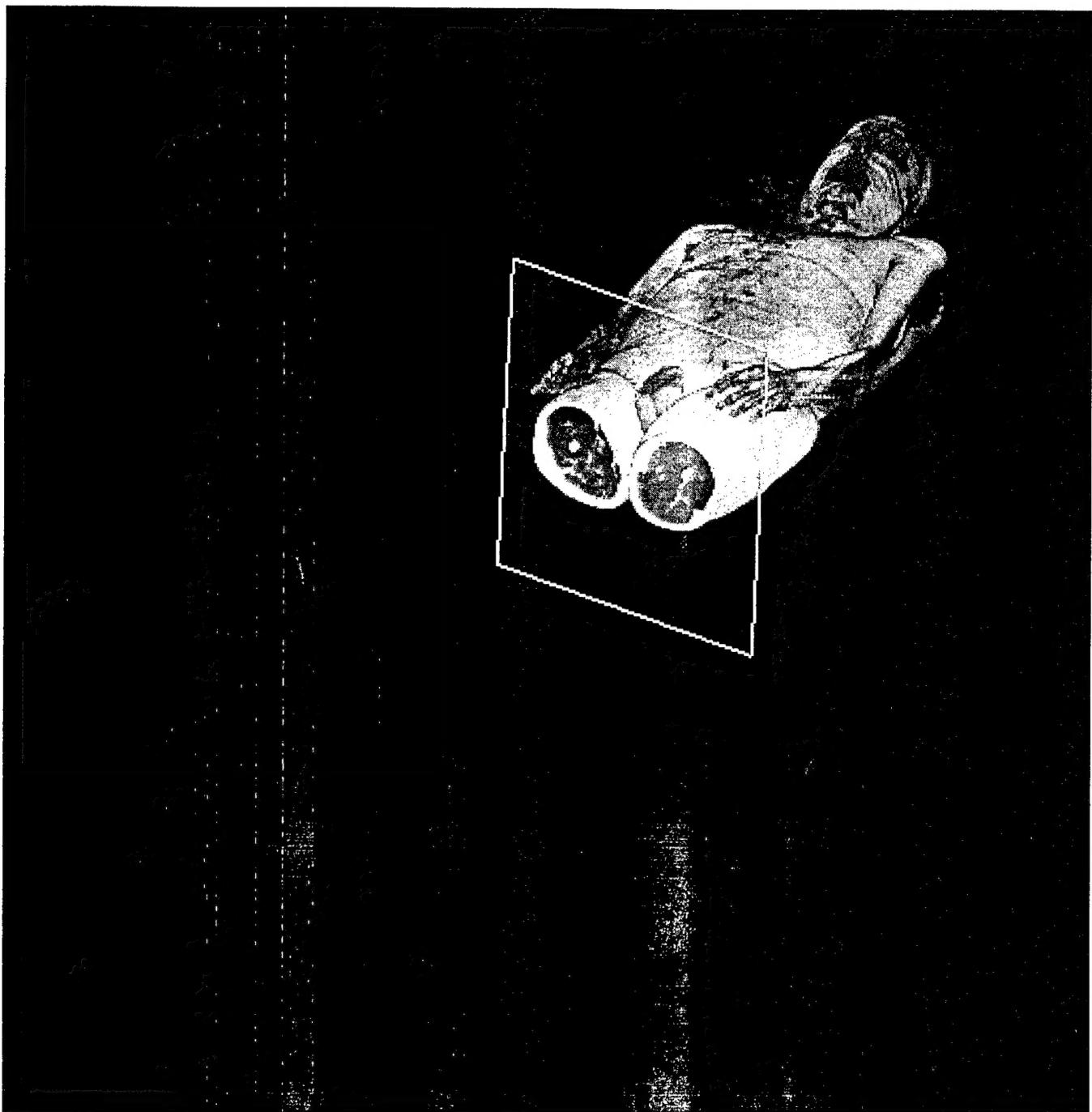


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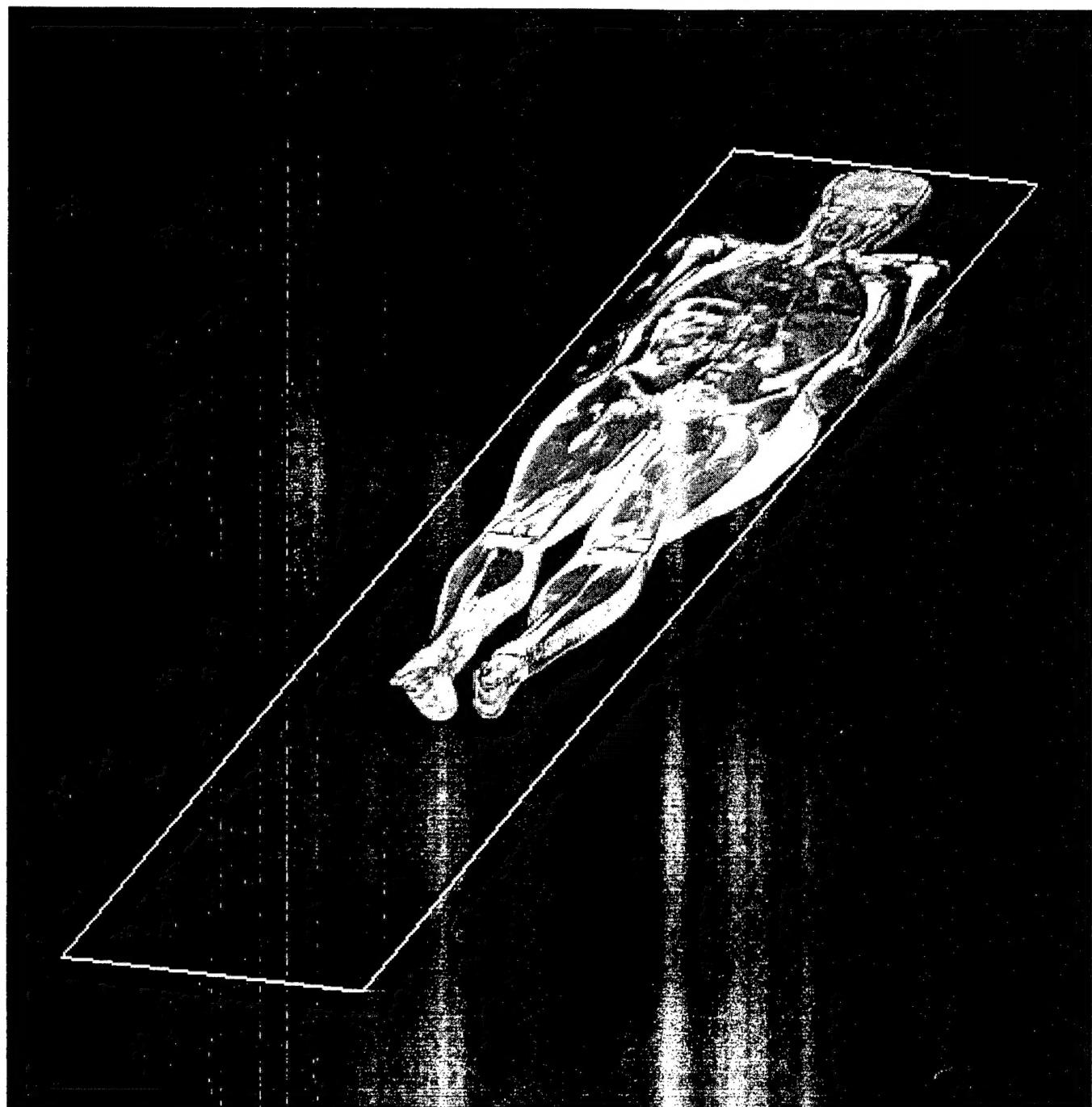


Figure 12:

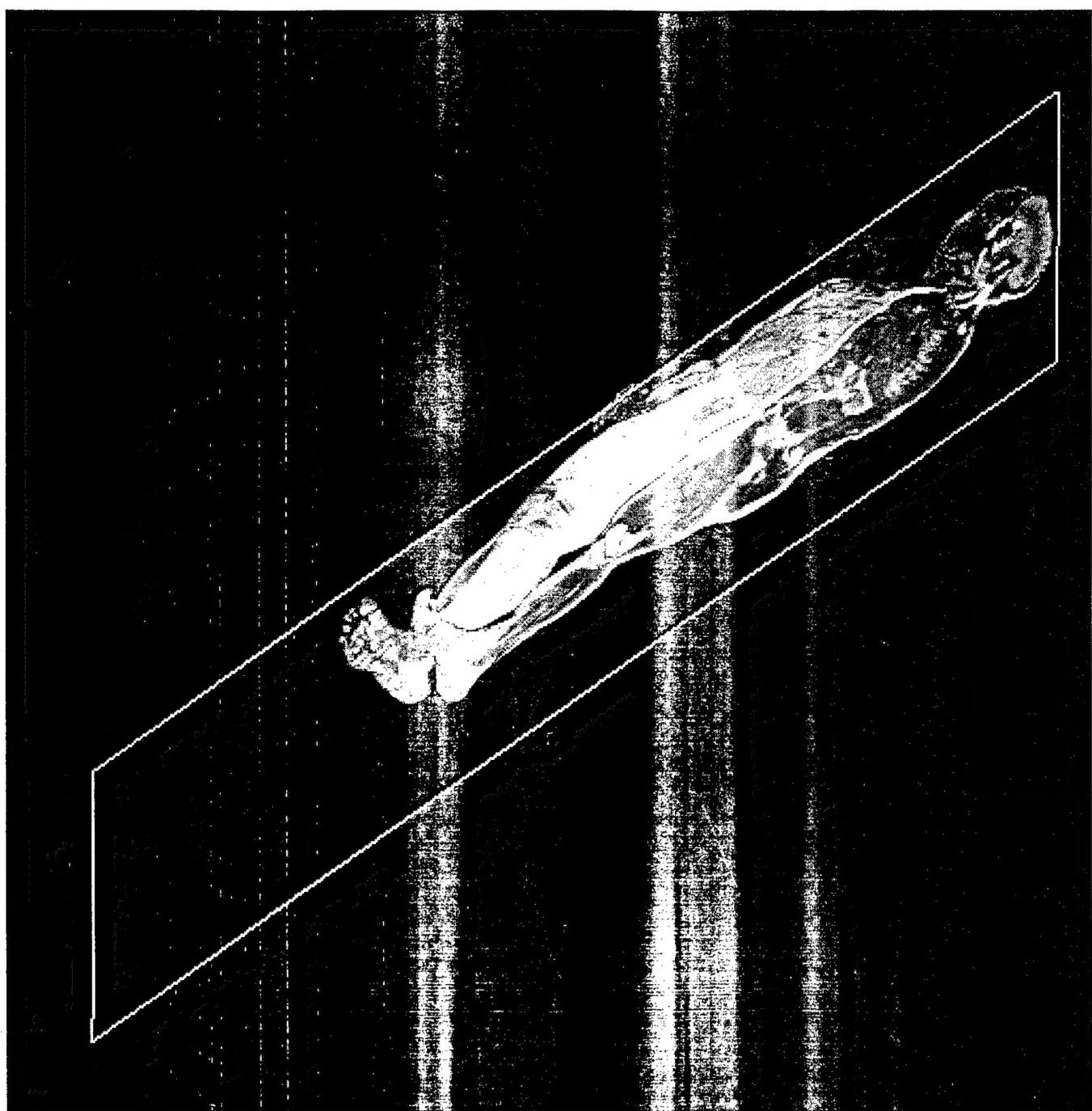


Figure 13 :

